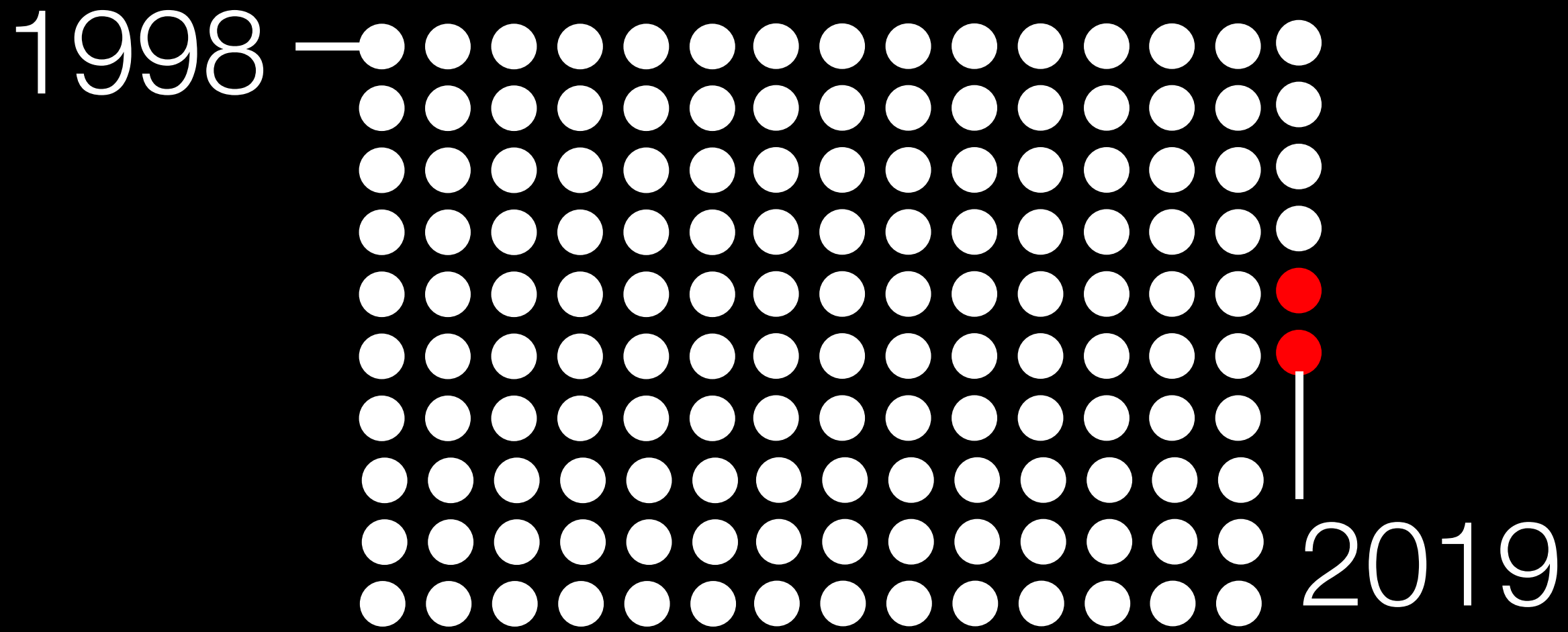
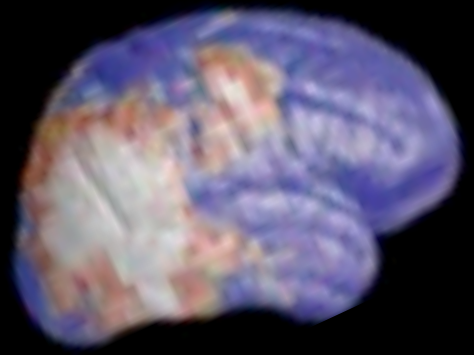
@nhihin

Alzheimer's disease drug trials:



Amyloid beta

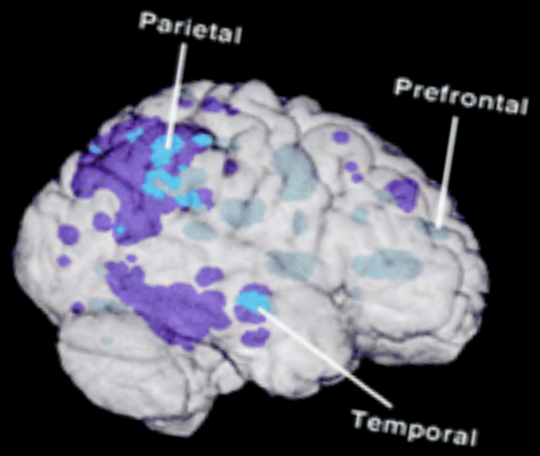
↓ **Cortical thickness**



MRI

Altered iron homeostasis?

↓ **Glucose metabolism**

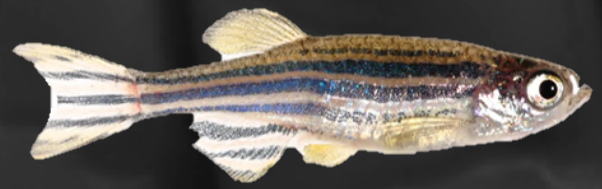


FDG-PET

Young adults with familial Alzheimer's disease

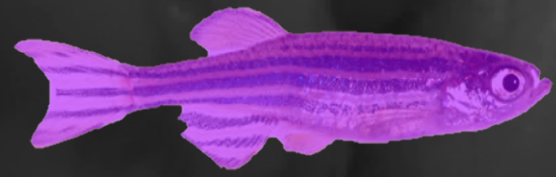
Apostolova et al. 2011; Reiman et al. 2004; Lumsden et al. 2018

RNA-seq analysis on whole-brains



Wild-type

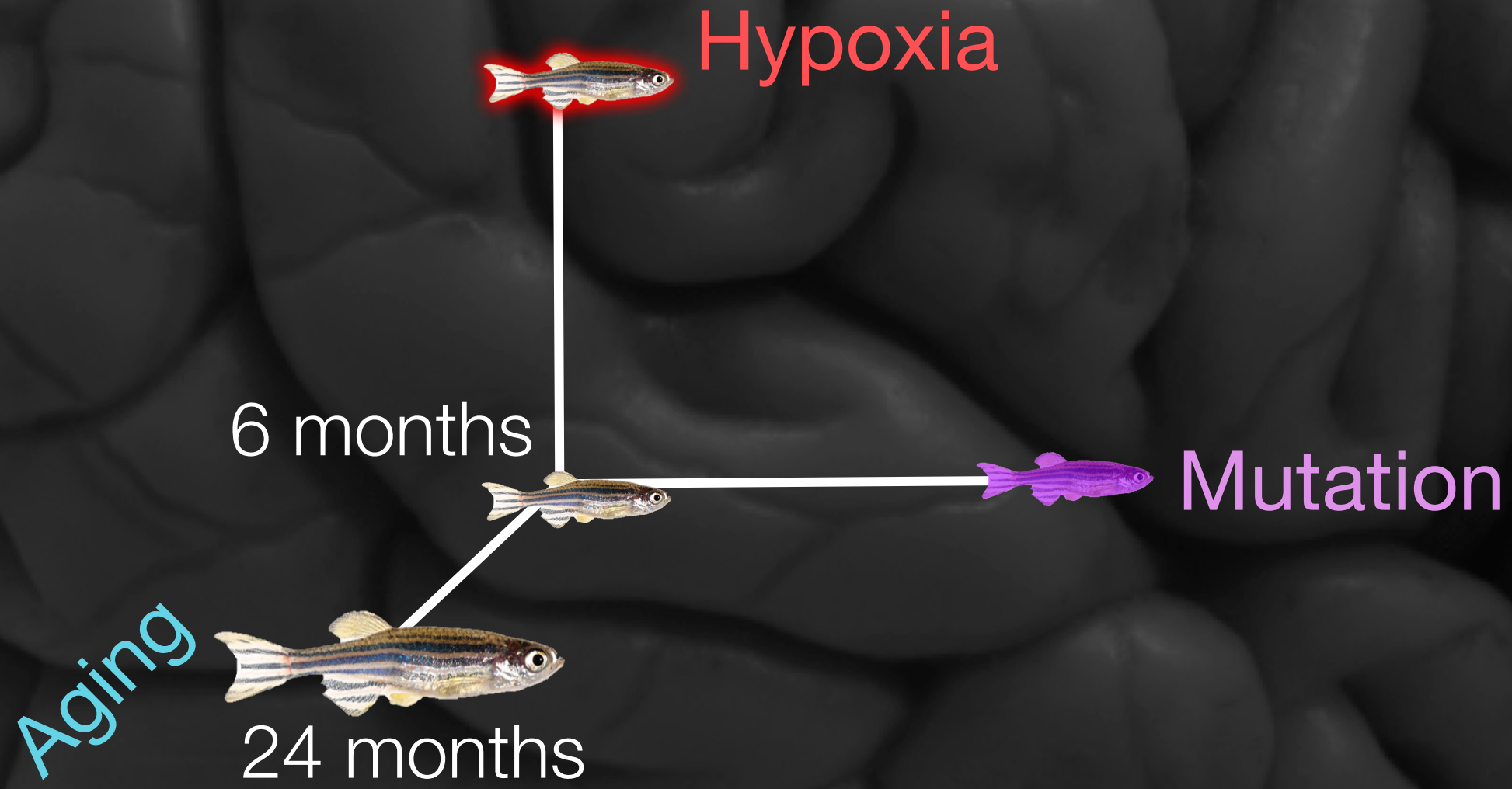
psen1$^{+/+}$



familial Alzheimer's-like

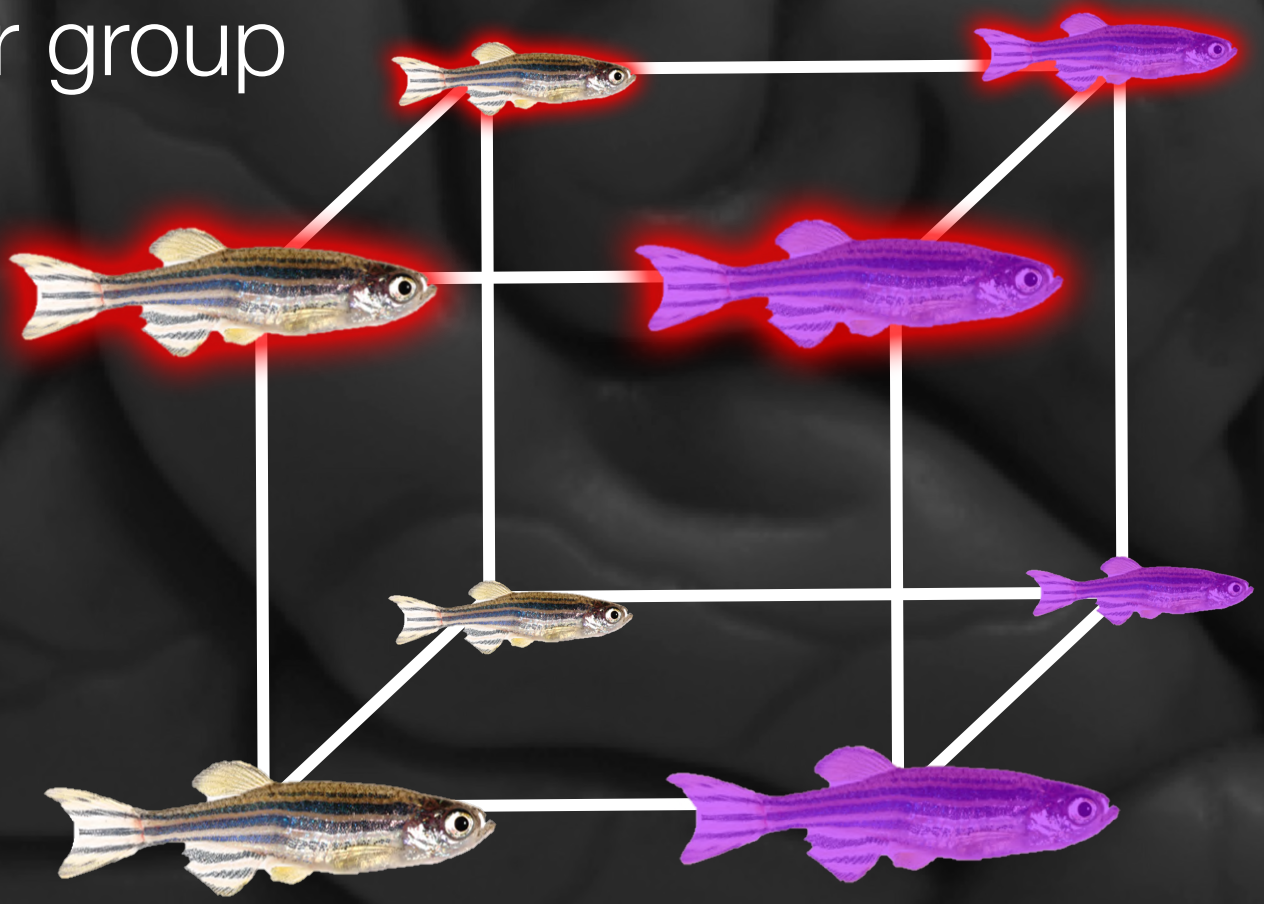
psen1$^{Q96_K97del/+}$

Experimental Design



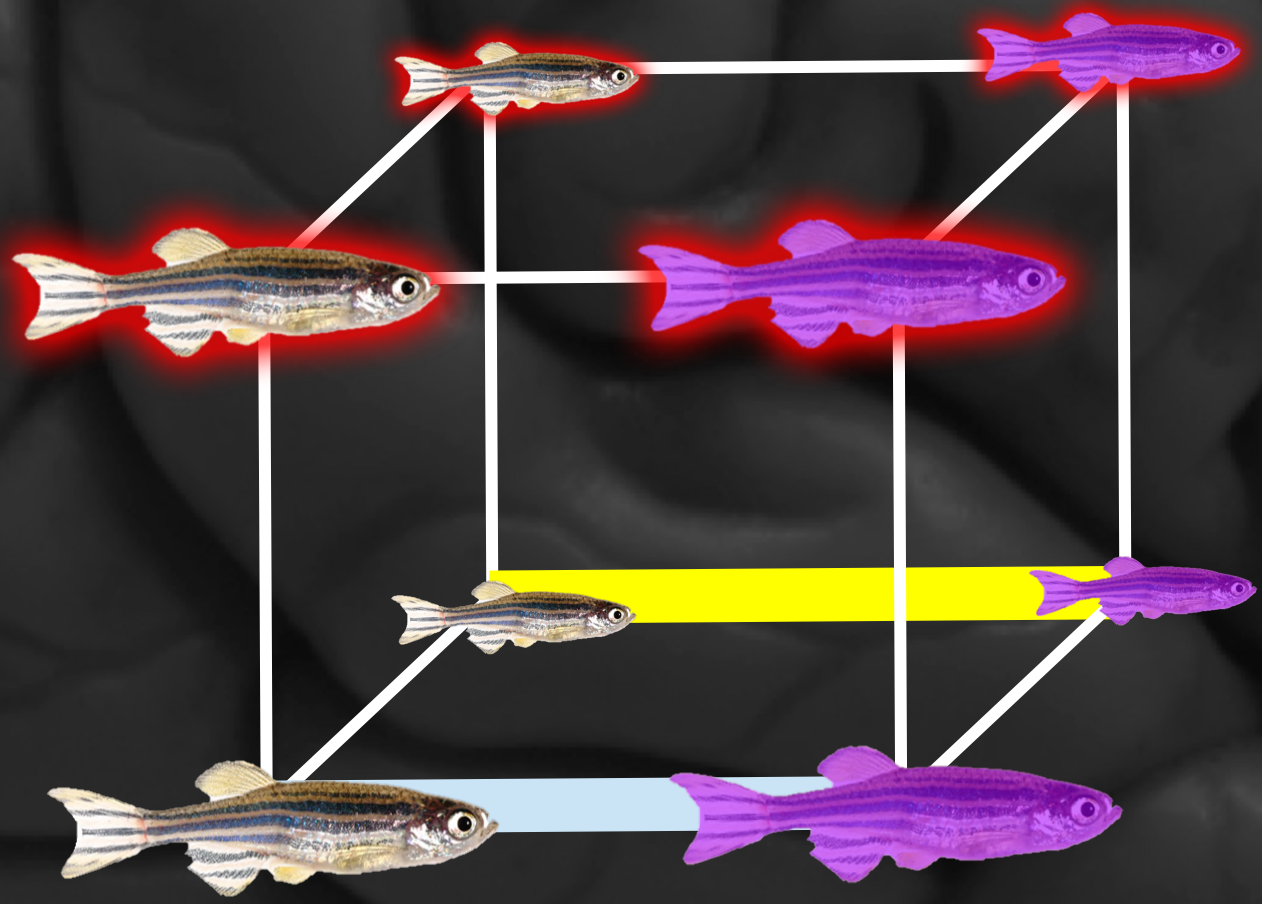
Experimental Design

n=4 per group



- Libraries prepared with whole-brains
- polyA-enriched
- single-end 75bp reads, Illumina NextSeq
- Quality trimmed
- Aligned to GRCz11with STAR
- FeatureCounts

1. Early changes in the brain before Alzheimer's disease?



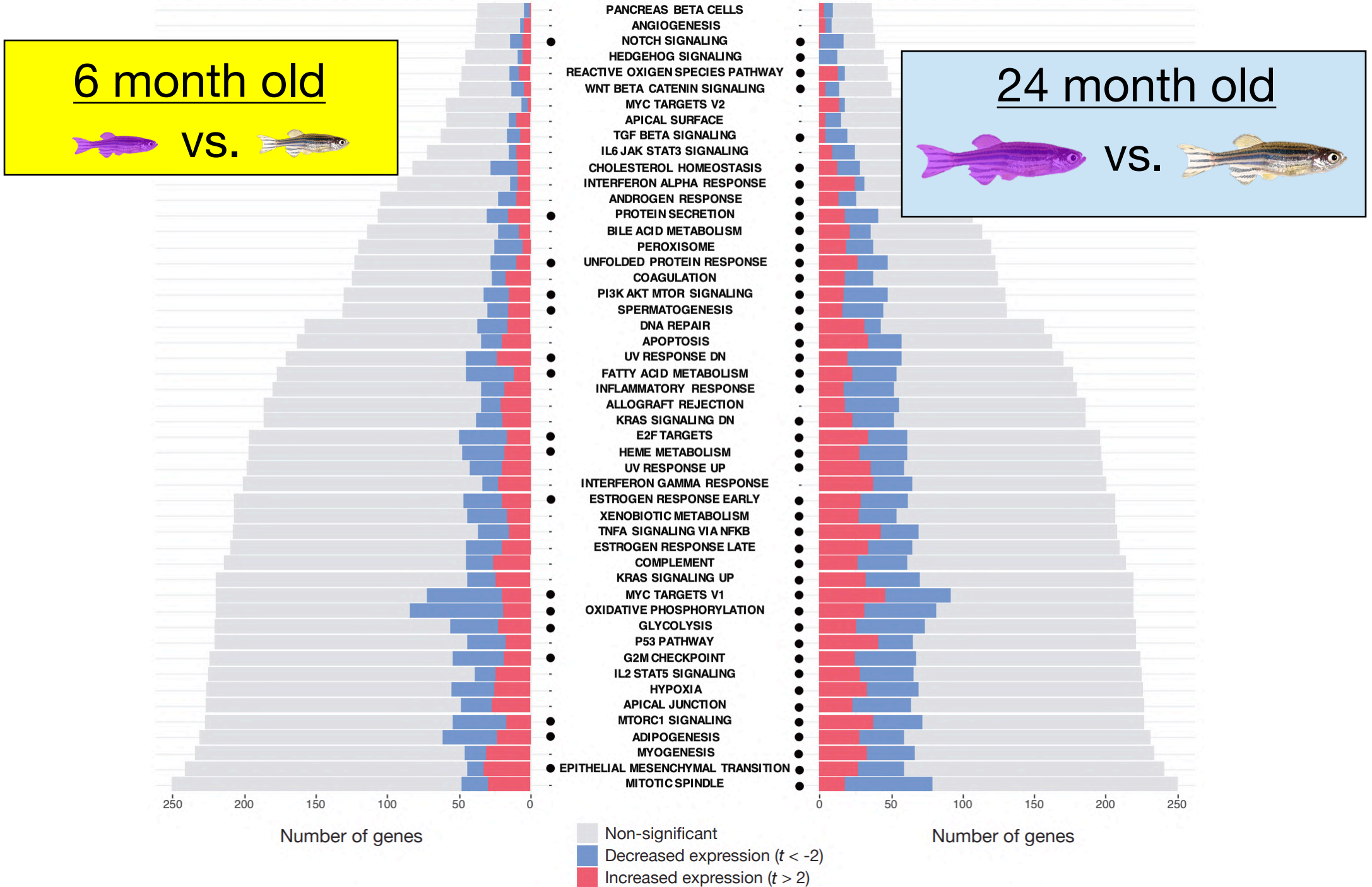
- *limma-voom* workflow
- *mroast, camera*, and *fgsea* for gene set analysis
- 50 Hallmark gene sets from MSigDB
- FDR adj. $p < 0.05$

6 month old

vs.

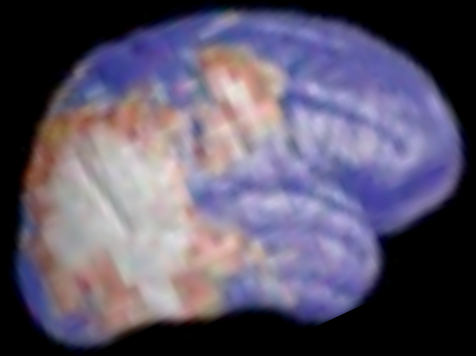
24 month old

vs.


6 month old
vs.
24 month old
vs.
PANCREAS BETA CELLS
ANGIOGENESIS
NOTCH SIGNALING
HEDGEHOG SIGNALING
REACTIVE OXIGEN SPECIES PATHWAY
WNT BETA CATENIN SIGNALING
MYC TARGETS V2
APICAL SURFACE
TGF BETA SIGNALING
IL6 JAK STAT3 SIGNALING
CHOLESTEROL HOMEOSTASIS
INTERFERON ALPHA RESPONSE
ANDROGEN RESPONSE
PROTEIN SECRETION
BILE ACID METABOLISM
PEROXISOME
UNFOLDED PROTEIN RESPONSE
COAGULATION
PI3K AKT MTOR SIGNALING
SPERMATOGENESIS
DNA REPAIR
APOPTOSIS
UV RESPONSE DN
FATTY ACID METABOLISM
INFLAMMATORY RESPONSE
ALLOGRAFT REJECTION
KRAS SIGNALING DN
E2F TARGETS
HEME METABOLISM
UV RESPONSE UP
INTERFERON GAMMA RESPONSE
ESTROGEN RESPONSE EARLY
XENOBIOTIC METABOLISM
TNFA SIGNALING VIA NFKB
ESTROGEN RESPONSE LATE
COMPLEMENT
KRAS SIGNALING UP
MYC TARGETS V1
OXIDATIVE PHOSPHORYLATION
GLYCOLYSIS
P53 PATHWAY
G2M CHECKPOINT
IL2 STAT5 SIGNALING
HYPOXIA
APICAL JUNCTION
MTORC1 SIGNALING
ADIPOGENESIS
MYOGENESIS
EPITHELIAL MESENCHYMAL TRANSITION
MITOTIC SPINDLE
250
200
150
100
50
0
0
50
100
150
200
250
Number of genes
Number of genes
Non-significant
Decreased expression (t < -2)
Increased expression (t > 2)

2. Altered iron homeostasis?

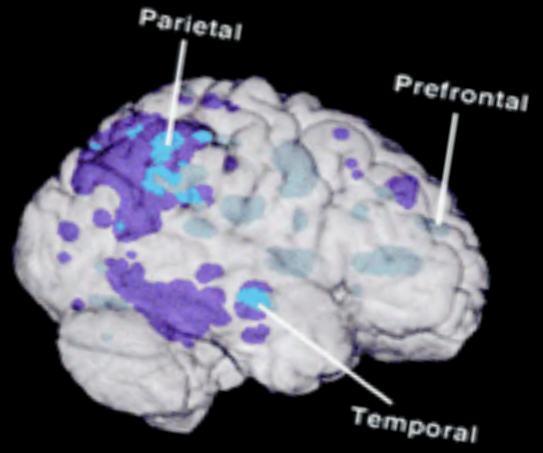
↓ **Cortical thickness**



MRI

Young adults with familial Alzheimer's disease

↓ **Glucose metabolism**



FDG-PET

Apostolova et al. 2011; Reiman et al. 2004

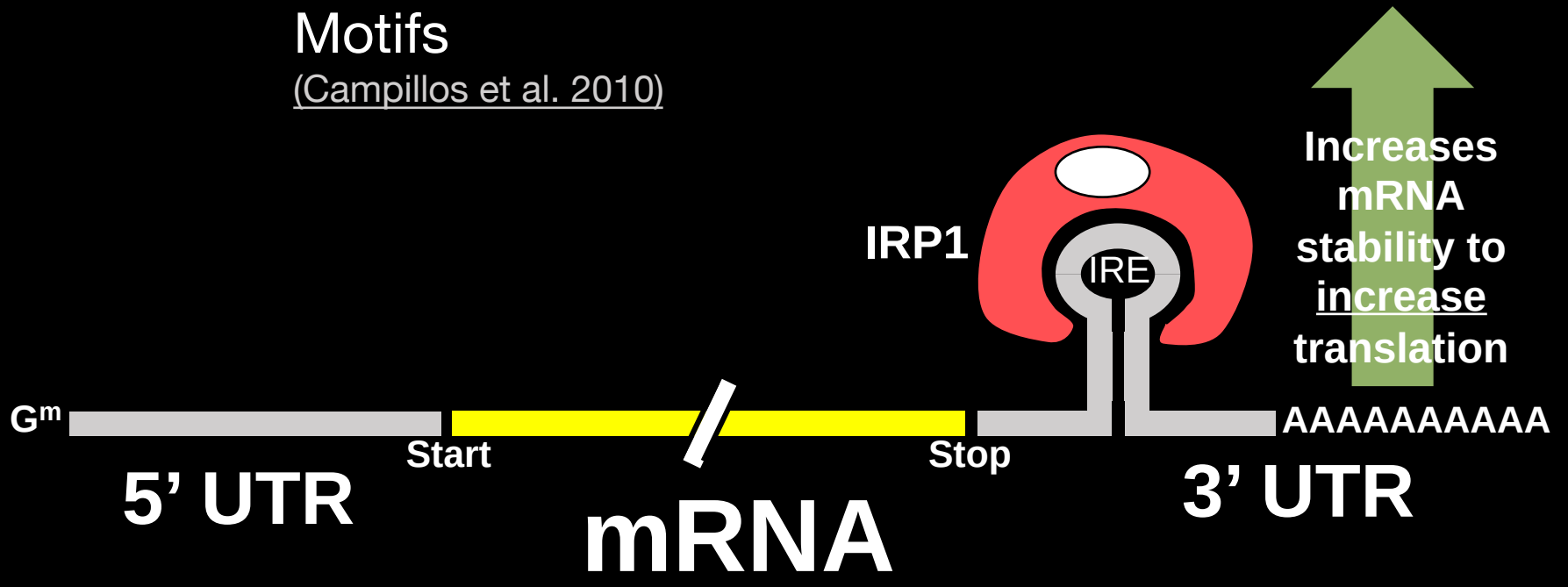
Searching for genes responding to disrupted iron homeostasis

Extract 3’ and 5’ UTR sequences From GRCz11

Search for IRE stem-loop Motifs (Campillos et al. 2010)

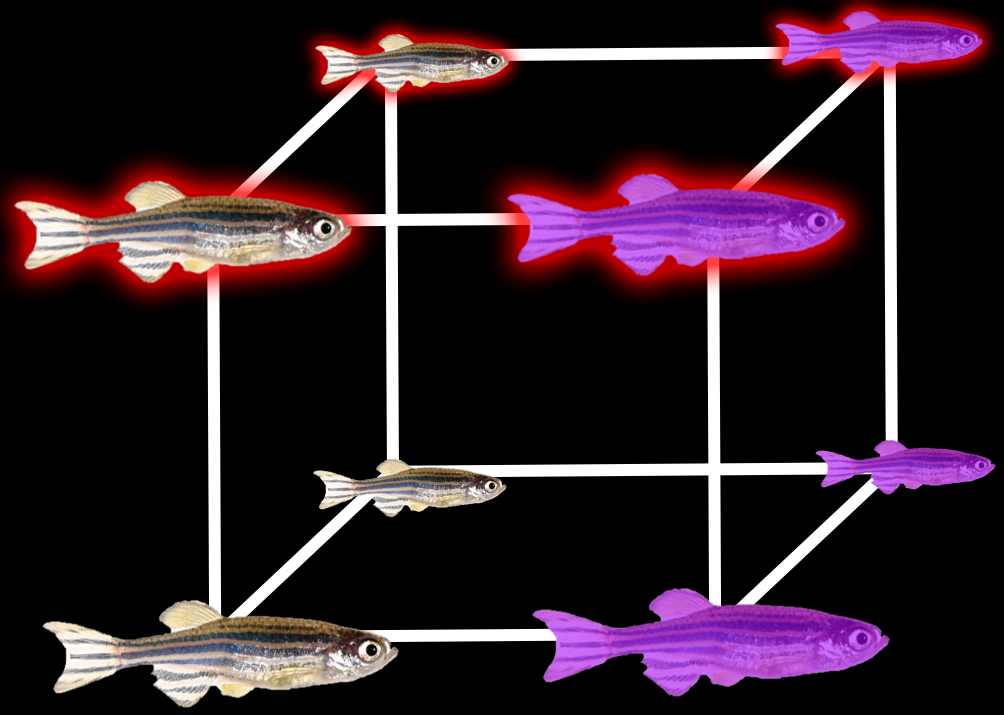
Zebrafish 3’ IRE gene set (1,207 genes)

Zebrafish 5’ IRE gene set (393 genes)



Zebrafish 3’
IRE gene set
(1,207 genes)

Zebrafish 5’
IRE gene set
(393 genes)

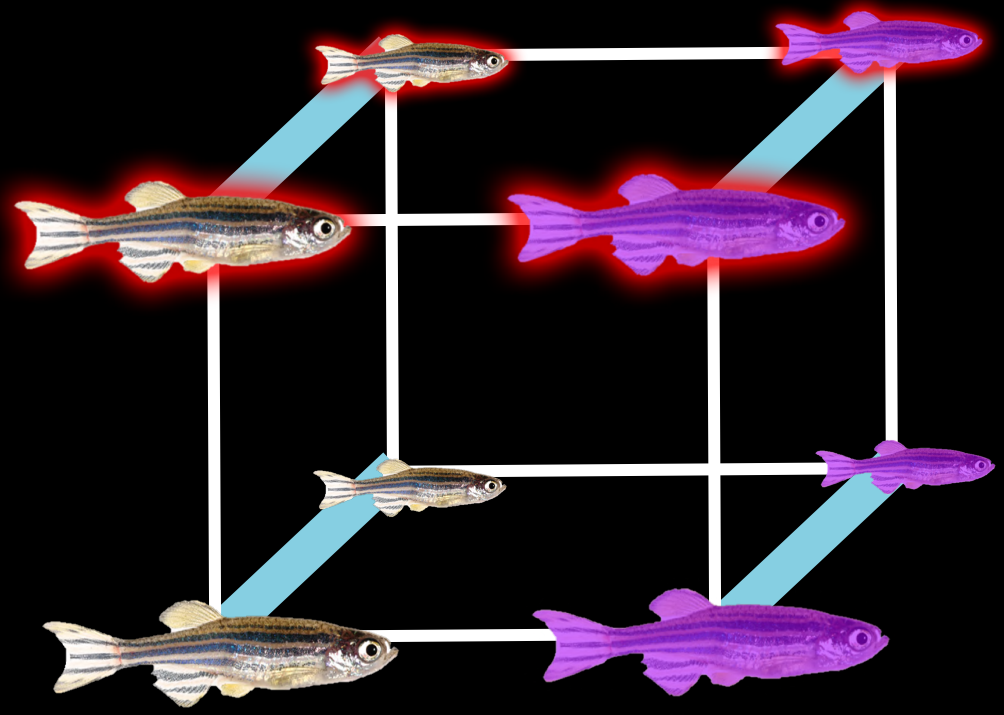


Gene set enrichment testing

mroast, camera, and fgsea, combined with Wilkinson’s method and FDR adj. $p < 0.05$

Zebrafish 3’
IRE gene set
(1,207 genes)

Zebrafish 5’
IRE gene set
(393 genes)



Gene set enrichment testing

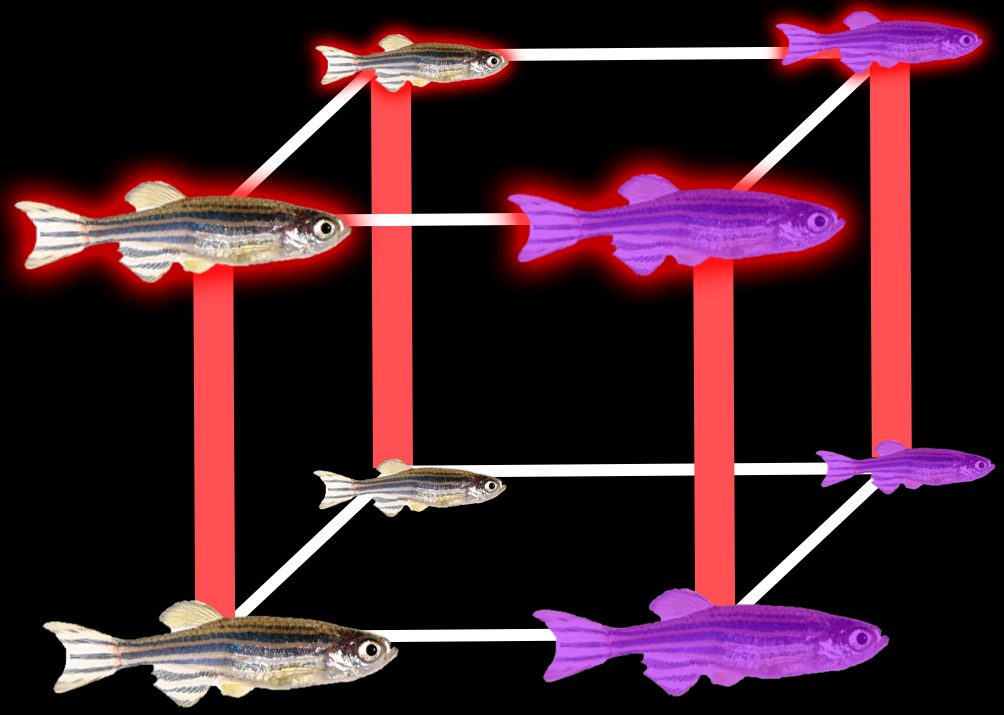
mroast, camera, and fgsea, combined with Wilkinson’s method and FDR adj. $p < 0.05$

Brain aging:

3’ and 5’ IRE gene set significantly enriched

Zebrafish 3’
IRE gene set
(1,207 genes)

Zebrafish 5’
IRE gene set
(393 genes)



Gene set enrichment testing

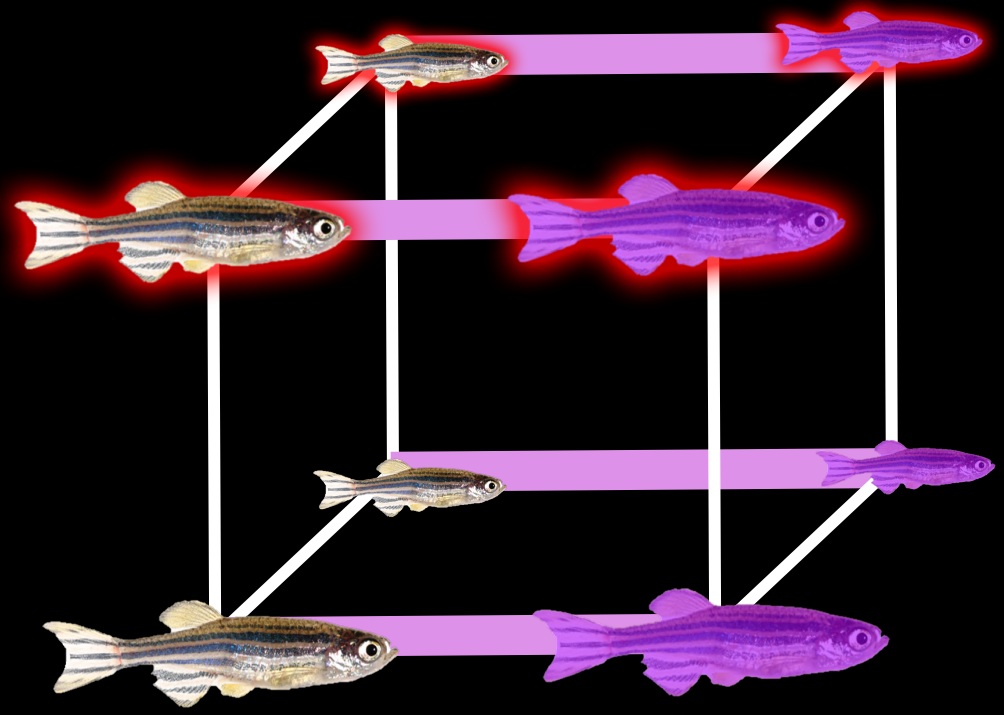
mroast, camera, and fgsea, combined with Wilkinson’s method and FDR adj. $p < 0.05$

Hypoxia (oxygen deficiency):

3’ and 5’ IRE gene set significantly enriched

Zebrafish 3’
IRE gene set
(1,207 genes)

Zebrafish 5’
IRE gene set
(393 genes)



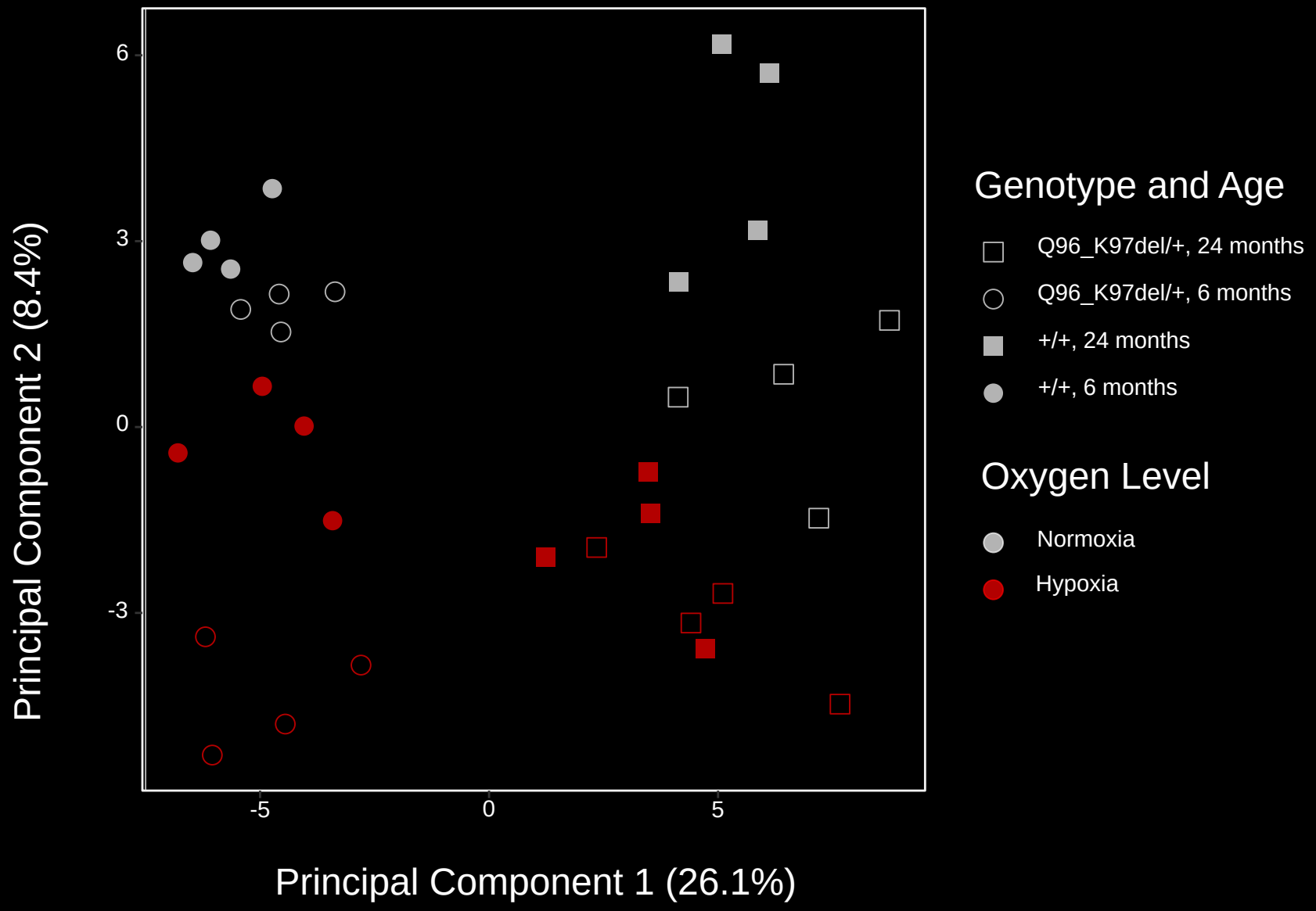
Gene set enrichment testing

mroast, camera, and fgsea, combined with Wilkinson’s method and FDR adj. $p < 0.05$

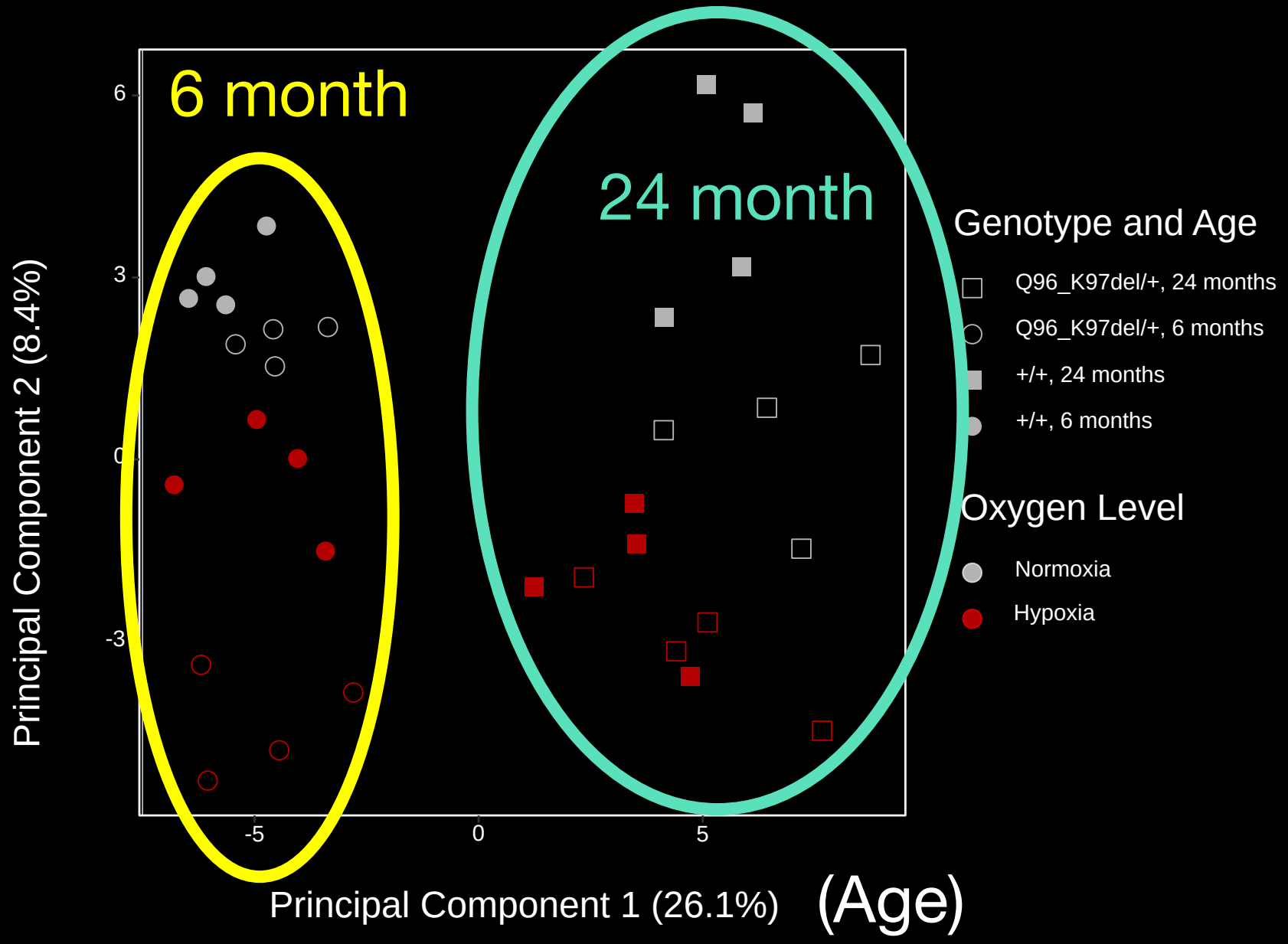
Alzheimer’s-like Mutation:

3’ IRE gene set significantly enriched

Expression of 1,207 3’ IRE genes across all samples

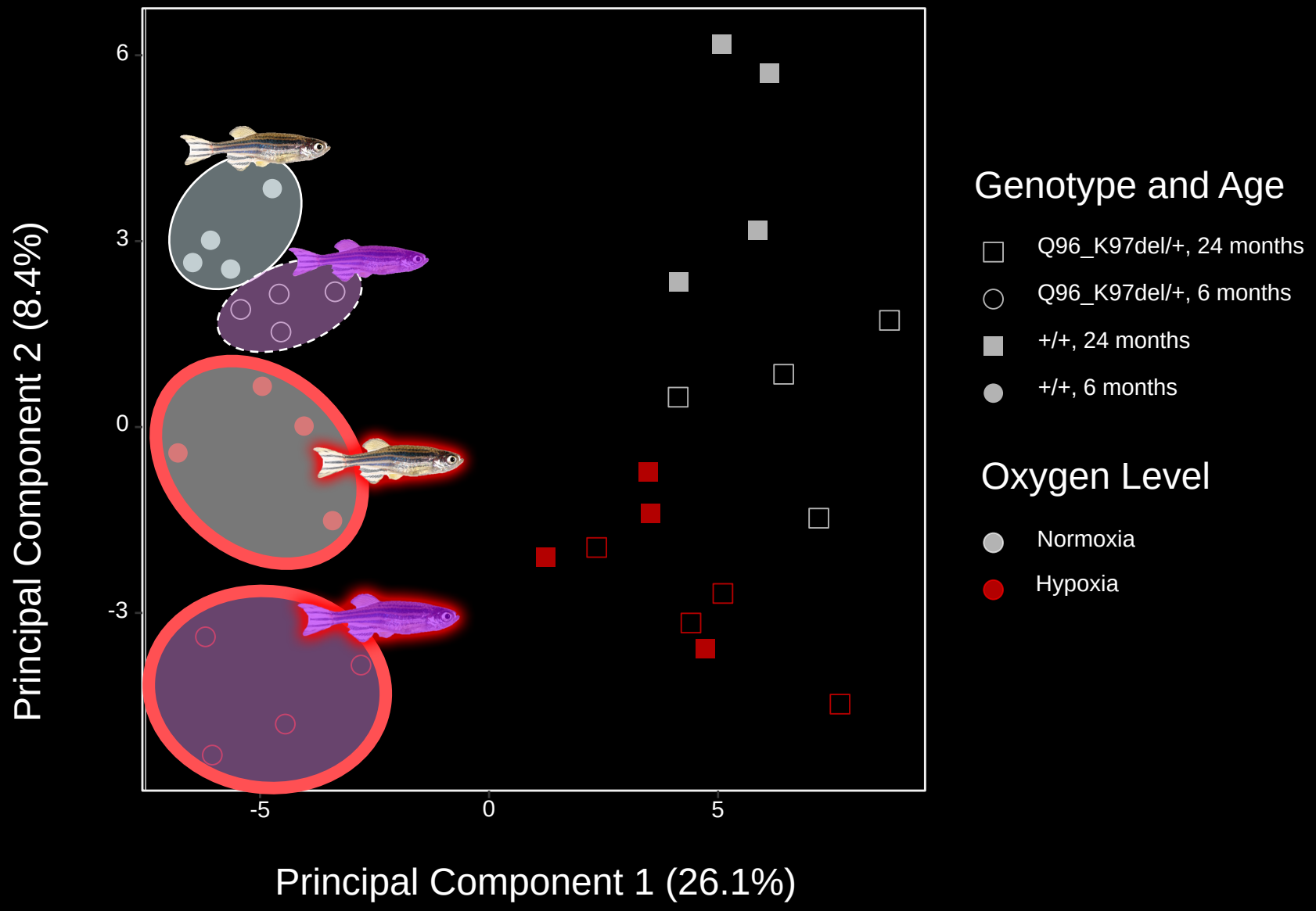


Expression of 1,207 3’ IRE genes across all samples

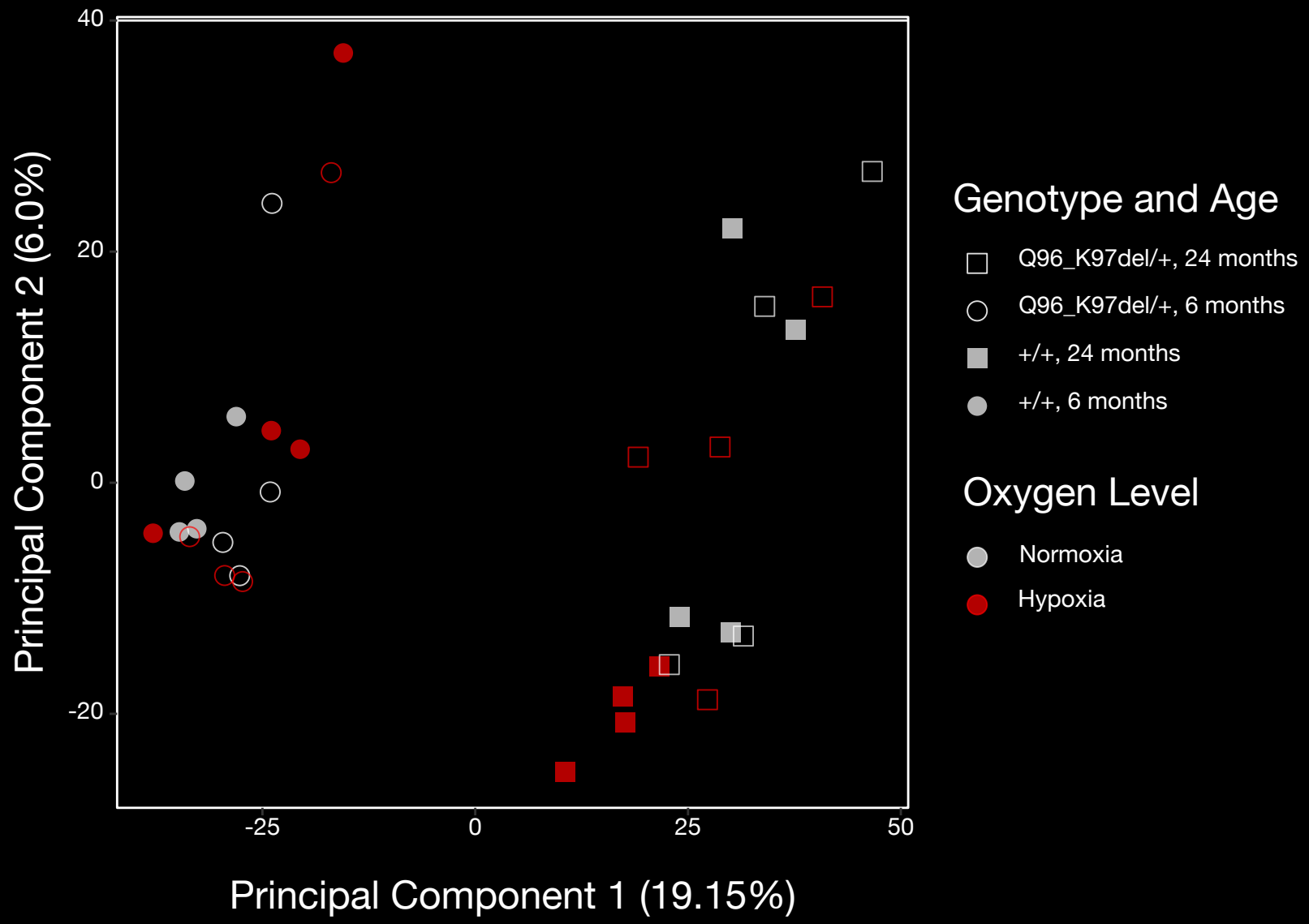


Expression of 1,207 3’ IRE genes across all samples

Hypoxia?

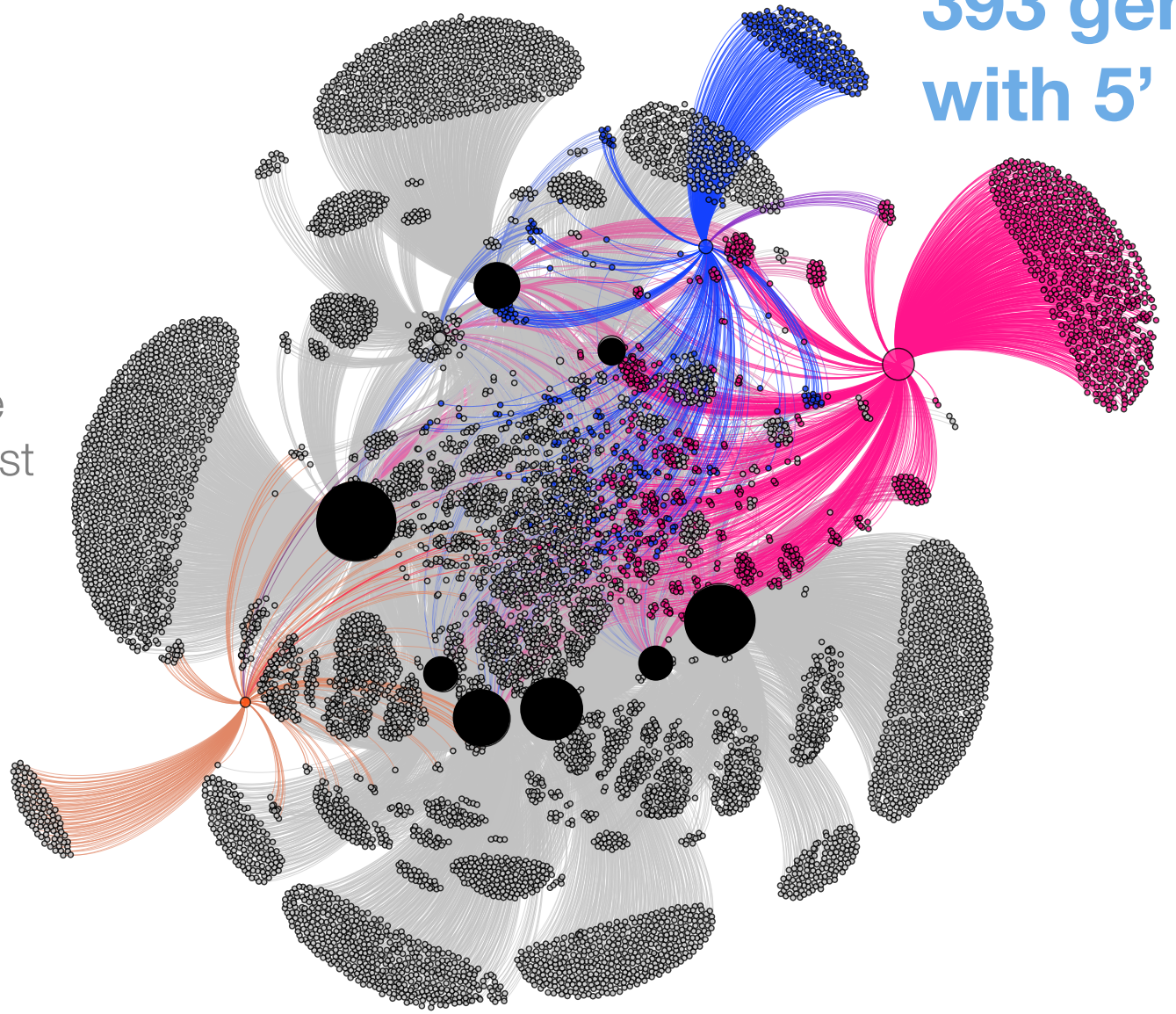


All genes


40
20
0
-20
-25
0
25
50
Principal Component 2 (6.0%)
Principal Component 1 (19.15%)
Genotype and Age
Q96_K97del/+, 24 months
Q96_K97del/+, 6 months
+/+, 24 months
+/+, 6 months
Oxygen Level
Normoxia
Hypoxia

393 genes
with 5’ IREs

Other gene sets from MSigDB with sig. overlap with IRE gene sets (Fisher’s exact test over-representation FDR p-value < 0.1)



1,207 genes
with 3’ IREs

Hallmark Heme Metabolism (200 genes)

Summary

- RNA-seq analysis in a zebrafish model of familial Alzheimer's disease has given us insight into potential early disease-causing changes in the brain.

- Genes with Iron Responsive Elements can give us more information about iron homeostasis than existing gene sets.
  - Revealing possibly shared mechanisms in familial Alzheimer's disease and hypoxia

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